PATENT COOPERATION T ATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 June 2000 (13.06.00)	
International application No. PCT/US99/23764	**Applicant's or agent's file reference** DEX-0048
International filing date (day/month/year) 18 October 1999 (18.10.99)	**Priority date** (day/month/year) 19 October 1998 (19.10.98)
Applicant ALI, Shujath, M. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 May 2000 (09.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

WRITTEN OPINION

(PCT Rule 66)

To: JANE MASSEY LICATA
LAW OFFICES OF JANE MASSEY LICATA
66 E. MAIN STREET
MARLTON, NEW JERSEY 08053 US

Docket System √
Status Report √
Docket Book √

10/16/00 ANS

Date of Mailing *(day/month/year)*	16 AUG 2000
Applicant's or agent's file reference: DEX-0048	REPLY DUE within TWO months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23764	18 OCTOBER 1999	19 OCTOBER 1998

International Patent Classification (IPC) or both national classification and IPC
Please See Supplemental Sheet.

Applicant
DIADEXUS LLC

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 19 FEBRUARY 2001.

Name and mailing address of the IPEA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer
GEETHA P. BANSAL

Telephone No. (703) 308-0196

WRITTEN OPINION

International application No.
PCT/US99/23764

I. Basis of the opinion

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-24, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the claims:
pages 25-27, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*

WRITTEN OPINION

International application No.
PCT/US99/23764

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	7-11	YES
	Claims	1-6	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-11	NO
Industrial Applicability (IA)	Claims	1-11	YES
	Claims	NONE	NO

2. citations and explanations

Claims 1-6 lack novelty under PCT Article 33(2) as being anticipated by Olsson et al (1997) and Cho-Chung et al (1993).

The claims are drawn to methids of diagnosing, staging and monitoring the metastatic potential of prostate cancer by identifying or detecting an increase in CSGs or cancer specific genes, in a patient. The claims are also drawn to a method of identifying therapeutic agents that bind to CSGs. Olsson et al teach the diagnosis, staging, monitoring metastasis of prostate cancer. Olsson et al teach that tumor cells exhibit abnormally rearanged or mutated genes that are not present in normal cells (these can be considered cancer specific genes). Olsson et al teach RT-PCR as well as quantitative RT-PCR technology as applicable to the above mentioned methods, and applying the technology to various tissue and body fluid samples.
Cho-Chung et al teach the use of nucleic acid in the therapy of neoplasia with specific referene to anti-sense oligonucletides. The disclosure of Cho-Chung et al inherently teaches a method of identifying potential candidates that will bind to the CSGs in prostate cancer.

Claims 7-11 lack an inventive step under PCT Article 33(3) as being obvious over Olsson et al (1997) and Cho-Chung et al (1993).The claims are drawn to methids of diagnosing, staging and monitoring the metastatic potential of prostate cancer by identifying or detecting an increase in CSGs or cancer specific genes, in a patient wherein the CSGs comprise the SEQ IDs recited in the claims. The claims are also drawn to a method of identifying therapeutic agents that bind to the said CSGs. Olsson et al teach the diagnosis, staging, monitoring metastasis of prostate cancer. Olsson et al teach that tumor cells exhibit abnormally rearanged or mutated genes that are not present in normal cells (these can be considered cancer specific genes). Olsson et al teach RT-PCR as well as quantitative RT-PCR technology as applicable to the above mentioned methods, and applying the technology to various tissue and body fluid samples.
Cho-Chung et al teach the use of nucleic acid in the therapy of neoplasia with specific referene to anti-sense oligonucletides.
(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): A61K 39/395, 48/00; C12P/ 19/34; C12Q 1/68; GO1N 33/53, 33/574, 33/546, 33/567 and US Cl.: 424/130.1, 141.1, 155.1, 183.1; 435/6, 7.1, 7.23, 7.9, 91.2; 436/501, 504, 505, 547; 514/44; 536/ 23.5

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
The disclosure of Cho-Chung et al inherently teaches a method of identifying potential candidates that will bind to the CSGs in prostate cancer. Though the above cited art do not specifically mention the SEQ ID Nos, it would have been obvious to one of ordinary skill in the art at the time of the invention to extend the methods wherein the PSA or PMSA have been replaced with the SEQ ID NOs recited in the claims. One of ordinary skill in the art would have been motivated to do so because the prior art teaches that cancer specific genes include any genes that are expressed by cancer cells and not by normal cells. Absent any unexpected results by the inclusion of the SEQ ID Nos recited in the claims , the use of these sequences in a method of diagnosing, staging and monitoring the metastatic potential of prostate cancer would have been obvious to do for one of ordinary skill ion the art. Furthermore, it would have been obvious to one of ordinary skill in the art to have produced antibodies to the CSGs as the art of making antibodies to peptides, proteins or other biochemica, molecules are routine in the art. The use of antibodies to the CSGs as an immunotoxin or in methods of detection would also have been obvious.

---------- NEW CITATIONS ----------
NONE

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference DEX-0048	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/23764	International filing date *(day/month/year)* 18 OCTOBER 1999	Priority date *(day/month/year)* 19 OCTOBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant DIADEXUS LLC		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09 MAY 2000	19 JANUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GEETHA P. BANSAL Telephone No. (703) 305-3955

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/23764

I. Basis f the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-24 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 25-27 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

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[ ] contained in the international application in printed form.

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[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reas ned statement under Article 35(2) with regard t n velty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	7-11	YES
	Claims	1-6	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-11	NO
Industrial Applicability (IA)	Claims	1-11	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-6 lack novelty under PCT Article 33(2) as being anticipated by Olsson et al (1997) and Cho-Chung et al (1993).

The claims are drawn to methids of diagnosing, staging and monitoring the metastatic potential of prostate cancer by identifying or detecting an increase in CSGs or cancer specific genes, in a patient. The claims are also drawn to a method of identifying therapeutic agents that bind to CSGs. Olsson et al teach the diagnosis, staging, monitoring metastasis of prostate cancer. Olsson et al teach that tumor cells exhibit abnormally rearanged or mutated genes that are not present in normal cells (these can be considered cancer specific genes). Olsson et al teach RT-PCR as well as quantitative RT-PCR technology as applicable to the above mentioned methods, and applying the technology to various tissue and body fluid samples.
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Cho-Chung et al teach the use of nucleic acid in the therapy of neoplasia with specific referene to anti-sense oligonucletides.
(Continued on Supplemental Sheet.)

Supplemental B x
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): A61K 39/395, 48/00; C12P/ 19/34; C12Q 1/68; GO1N 33/53, 33/574, 33/546, 33/567 and US Cl.: 424/130.1, 141.1, 155.1, 183.1; 435/6, 7.1, 7.23, 7.9, 91.2; 436/501, 504, 505, 547; 514/44; 536/ 23.5

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

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---------- NEW CITATIONS ----------

NONE

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 39/395, 48/00, C12P 19/34, C12Q 1/68, G01N 33/53, 33/574, 33/546, 33/567

A1

(11) International Publication Number: **WO 00/23108**

(43) International Publication Date: 27 April 2000 (27.04.00)

(21) International Application Number: PCT/US99/23764

(22) International Filing Date: 18 October 1999 (18.10.99)

(30) Priority Data:
60/104,741 19 October 1998 (19.10.98) US

(71) Applicant *(for all designated States except US)*: DIADEXUS LLC [US/US]; 3303 Octavius Drive, Santa Clara, CA 95054 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ALI, Shujath, M. [IN/US]; 3475 Granada Avenue, #357, Santa Clara, CA 95051 (US). SUN, Yongming [CN/US]; Apartment 260, 869 S. Winchester Boulevard, San Jose, CA 95128 (US). SALCEDA, Susana [AR/US]; 4118 Crescendo Avenue, San Jose, CA 95136 (US). RECIPON, Herve [FR/US]; 85 Fortuna Avenue, San Francisco, CA 94115 (US). CAFFERKEY, Robert [IE/US]; Apartment #218, 350 Elan Village Lane, San Jose, CA 95134 (US).

(74) Agents: LICATA, Jane, Massey et al.; Law Offices of Jane Massey Licata, 66 E. Main Street, Marlton, NJ 08053 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD OF DIAGNOSING, MONITORING, STAGING, IMAGING AND TREATING PROSTATE CANCER

(57) Abstract

The present invention provides a new method for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating prostate cancer.

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METHOD OF DIAGNOSING, MONITORING, STAGING, IMAGING AND TREATING PROSTATE CANCER

FIELD OF THE INVENTION

This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating cancers, particularly prostate cancer.

BACKGROUND OF THE INVENTION

Cancer of the prostate is the most prevalent malignancy in adult males, excluding skin cancer, and is an increasingly prevalent health problem in the United States. In 1996, it was estimated that 41,400 deaths would result from this disease in the United States alone, indicating that prostate cancer is second only to lung cancer as the most common cause of death in the same population. If diagnosed and treated early, when the cancer is still confined to the prostate, the chances of cure is significantly higher.

Treatment decisions for an individual are linked to the stage of prostate cancer present in that individual. A common classification of the spread of prostate cancer was developed by the American Urological Association (AUA). The AUA system divides prostate tumors into four stages, A to D. Stage A, microscopic cancer within prostate, is further subdivided into sub-stages A1 and A2. Sub-stage A1 is a well-differentiated cancer confined to one site within the prostate. Treatment is generally observation, radical prostatectomy, or radiation. Sub-stage A2 is a moderately to poorly differentiated cancer at multiple sites within the prostate. Treatment is radical prostatectomy or radiation. Stage B, palpable lump within the

prostate, is also further subdivided into sub-stages B1 and B2. In sub-stage B1, the cancer forms a small nodule in one lobe of the prostate. In sub-stage B2, the cancer forms large or multiple nodules, or occurs in both lobes of the prostate. Treatment for sub-stages B1 and B2 is either radical prostatectomy or radiation. Stage C is a large cancer mass involving most or all of the prostate and is also further subdivided into two stages. In sub-stage C1, the cancer forms a continuous mass that may have extended beyond the prostate. In sub-stage C2, the cancer forms a continuous mass that invades the surrounding tissue. Treatment for both these sub-stages is radiation with or without drugs to address the cancer. The fourth stage, Stage D is metastatic cancer and is also subdivided into two sub-stages. In sub-stage D1, the cancer appears in the lymph nodes of the pelvis. In sub-stage D2, the cancer involves tissues beyond lymph nodes. Treatment for both of these sub-stages is systemic drugs to address the cancer as well as pain.

However, current prostate cancer staging methods are limited. As many as 50% of prostate cancers initially staged as A2, B, or C are actually stage D, metastatic. Discovery of metastasis is significant because patients with metastatic cancers have a poorer prognosis and require significantly different therapy than those with localized cancers. The five year survival rates for patients with localized and metastatic prostate cancers are 93% and 29%, respectively.

Accordingly, there is a great need for increasingly sensitive methods for diagnosing and staging of prostate cancer in a human patient to determine whether or not such cancer has metastasized and for monitoring the progress of a cancer which has not metastasized for the onset of metastasis.

In the present invention, methods are provided for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating prostate cancer via the cancer specific gene referred to herein as CSG. CSG refers, among other things, to native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. In the alternative, what is meant by CSG as used herein, means the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of prostate cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein a change in levels of

CSG in the patient versus the normal human control is associated with prostate cancer.

Further provided is a method of diagnosing metastatic prostate cancer in a patient having prostate cancer which is not known to have metastasized by identifying a human patient suspected of having prostate cancer that has metastasized; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in CSG levels in the patient versus the normal human control is associated with prostate cancer which has metastasized.

Also provided by the invention is a method of staging prostate cancer in a human which has such cancer by identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Further provided is a method of monitoring prostate cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in

preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided is a method of monitoring the change in stage of prostate cancer in a human having such cancer by looking at levels of CSG in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Further provided are antibodies targeted against CSG or fragments of such antibodies which can be used to detect or image localization of CSG in a patient for the purpose of detecting or diagnosing a disease or condition. Such antibodies can be polyclonal, monoclonal, or omniclonal or prepared by molecular biology techniques. The term "antibody", as used herein and throughout the instant specification is also meant to include aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art. Antibodies can be labeled with a variety of detectable labels including, but not limited to, radioisotopes and paramagnetic metals. These antibodies or fragments thereof can also be used as therapeutic agents in

the treatment of diseases characterized by expression of CSG. In therapeutic applications, the antibody can be used without or with derivatization to a cytotoxic agent such as a radioisotope, enzyme, toxin, drug or a prodrug.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging and prognosticating cancers by comparing levels of CSG in a human patient with those of CSG in a normal human control. What is meant by levels of CSG as used herein, means levels of the native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. The native protein being detected, may be whole, a breakdown product, a complex of molecules or chemically modified. In the alternative, what is meant by levels of CSG as used herein, means levels of the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of DNA comprising the polynucleotide sequence of SEQ ID NO:1. Such levels are

preferably measured in at least one of cells, tissues and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing over-expression of CSG protein compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of prostate cancer.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as CSG. Other cancer markers, in addition to CSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

Diagnostic Assays

The present invention provides methods for diagnosing the presence of prostate cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of CSG in the patient versus the normal human control is associated with the presence of prostate cancer.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing metastatic prostate cancer in a patient having prostate cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a

human cancer patient suspected of having prostate cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art.

In the present invention, determining the presence of CSG levels in cells, tissues or bodily fluid, is particularly useful for discriminating between prostate cancer which has not metastasized and prostate cancer which has metastasized. Existing techniques have difficulty discriminating between prostate cancer which has metastasized and prostate cancer which has not metastasized. However, proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissues or bodily fluid is CSG. Measured levels of CSG are compared with levels of CSG in preferably the same cells, tissue or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just CSG in serum, this level is preferably compared with the level of CSG in serum of a normal human patient. An increase in CSG levels in the patient versus the normal human control is associated with prostate cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a normal patient.

Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing or monitoring for metastasis, normal human control may preferably also include samples from a human patient that is determined by reliable methods to have prostate cancer which has not metastasized.

Staging

The invention also provides a method of staging prostate cancer in a human patient. The method comprises identifying a human patient having such cancer and analyzing a sample of cells, tissues or bodily fluid from such human patient for CSG. The measured CSG levels in the patient are then compared with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in CSG levels in the human patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG (but still increased over true normal levels) is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring prostate cancer in a human patient having such cancer for the onset of metastasis. The method comprises identifying a human patient having prostate cancer that is not known to have metastasized; periodically analyzing cells, tissues or bodily fluid from such human patient for CSG; comparing the CSG levels in such cells, tissues or bodily fluid with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in CSG levels in the human patient versus the normal human control is associated

with a cancer which has metastasized. In this method, normal human control samples may also include prior patient samples.

Further provided by this invention is a method of monitoring the change in stage of prostate cancer in a human patient having such cancer. The method comprises identifying a human patient having prostate cancer; periodically analyzing cells, tissues or bodily fluid from such human patient for CSG; comparing the CSG levels in such cells, tissues or bodily fluid with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in CSG levels in the human patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of CSG is associated with a cancer which is regressing in stage or in remission. In this method, normal human control samples may also include prior patient samples.

Monitoring patients for onset of metastasis is periodic and preferably done on a quarterly basis. However, monitoring may be performed more or less frequently depending on the cancer, the particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of gene expression (including protein levels), such as CSG of the present invention, in a sample derived from a patient are well known to those of skill in the art. Such assay methods include, without limitation, radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses, ELISA assays and proteomic approaches: two-dimensional gel electrophoresis (2D electrophoresis) and non-gel based approaches such as mass

spectrometry or protein interaction profiling. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.

An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to CSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to CSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent. For example, detectable agents such as horseradish peroxidase enzyme and alkaline phosphatase are routinely used in these types of assays.

To carry out the ELISA, antibody specific to CSG is incubated on a solid support, e.g. a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time CSG binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to CSG and linked to a detectable reagent such as horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to CSG. Unattached reporter antibody is then washed out. Reagents for detection of peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase linked to CSG antibodies produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of CSG protein present in the sample. Quantitative

results typically are obtained by reference to a standard curve.

A competition assay can also be employed wherein antibodies specific to CSG are attached to a solid support. Labeled CSG and a sample derived from the host are then passed over the solid support. The amount of label detected which is attached to the solid support can be correlated to a quantity of CSG in the sample.

Nucleic acid methods can be used to detect CSG mRNA as a marker for prostate cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (*i.e.* gridding) can be used to detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the CSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or

plastic. At least a portion of the DNA encoding the CSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including, but not limited to, radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

Of the proteomic approaches, 2D electrophoresis is a technique well known to those in the art. Isolation of individual proteins from a sample such as serum is accomplished using sequential separation of proteins by different characteristics usually on polyacrylamide gels. First, proteins are separated by size using an electric current. The current acts uniformly on all proteins so that smaller proteins move farther on the gel than larger proteins. The second dimension applies a current perpendicular to the first and separates proteins not on the basis of size but on the specific electric charge carried by each protein. Since no two proteins with different sequences are identical on the basis of both size and charge, the result of a 2D separation is a square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes or subsequent protein microsequencing can reveal the relative

abundance of a given protein and the identity of the proteins in the sample.

The above tests can be carried out on samples derived from a variety of cells, bodily fluids and/or tissue extracts including both homogenates or solubilized tissue obtained from a human patient. Tissue extracts can be obtained from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva or any other bodily secretion or derivative thereof. By blood it is meant to include whole blood, plasma, serum or any derivative of blood.

In Vivo Antibody Use

Antibodies which specifically bind to CSG can also be used *in vivo* in patients suspected of suffering from prostate cancer. Specifically, antibodies which specifically bind a CSG can be injected into a patient suspected of having prostate cancer for diagnostic and/or therapeutic purposes. The preparation and use of antibodies for *in vivo* diagnosis is well known in the art. For example, antibody-chelators labeled with Indium-111 have been described for use in the radioimmunoscintographic imaging of carcinoembryonic antigen expressing tumors (Sumerdon et al. Nucl. Med. Biol. 1990 17:247-254). In particular, these antibody-chelators have been used in detecting tumors in patients suspected of having recurrent colorectal cancer (Griffin et al. J. Clin. Onc. 1991 9:631-640). Antibodies with paramagnetic ions as labels for use in magnetic resonance imaging have also been described (Lauffer, R.B. Magnetic Resonance in Medicine 1991 22:339-342). Antibodies directed against CSG can be used in a similar manner. Labeled antibodies which specifically bind CSG can be injected into patients suspected of having prostate

cancer for the purpose of diagnosing or staging of the disease status of the patient. The label used will be selected in accordance with the imaging modality to be used. For example, radioactive labels such as Indium-111, Technetium-99m or Iodine-131 can be used for planar scans or single photon emission computed tomography (SPECT). Positron emitting labels such as Fluorine-19 can be used in positron emission tomography. Paramagnetic ions such as Gadlinium (III) or Manganese (II) can be used in magnetic resonance imaging (MRI). Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue.

For patients diagnosed with prostate cancer, injection of an antibody which specifically binds CSG can also have a therapeutic benefit. The antibody may exert its therapeutic effect alone. Alternatively, the antibody can be conjugated to a cytotoxic agent such as a drug, toxin or radionuclide to enhance its therapeutic effect. Drug monoclonal antibodies have been described in the art for example by Garnett and Baldwin, Cancer Research 1986 46:2407-2412. The use of toxins conjugated to monoclonal antibodies for the therapy of various cancers has also been described by Pastan et al. Cell 1986 47:641-648. Yttrium-90 labeled monoclonal antibodies have been described for maximization of dose delivered to the tumor while limiting toxicity to normal tissues (Goodwin and Meares Cancer Supplement 1997 80:2675-2680). Other cytotoxic radionuclides including, but not limited to Copper-67, Iodine-131 and Rhenium-186 can also be used for labeling of antibodies against CSG.

Antibodies which can be used in these *in vivo* methods include polyclonal, monoclonal and omniclonal antibodies and antibodies prepared via molecular biology techniques. Antibody fragments and aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art can also be used.

The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

EXAMPLES

The examples are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Example 1

Suppression subtractive hybridization (CLONTECH PCR-SELECT)

CLONTECH PCR-SELECT is a PCR based subtractive hybridization method designed to selectively enrich for cDNAs corresponding to mRNAs differentially expressed between two mRNA populations (Diatchenko *et al, Proc. Natl. Acad. Sci. USA*, Vol. 93, pp. 6025-6030, 1996). In this method, differentially expressed mRNAs are enriched based on a selective amplification. cDNA is prepared from the two mRNA

populations which are to be compared. These include the Tester population which is a cDNA population in which the differentially expressed messages are sought and the Driver population which is a cDNA population in which the differentially expressed transcripts are absent or low. The tester sample is separated in two parts and different PCR adapters are ligated to the 5' ends. Each tester is separately annealed to excess driver in the first annealing and then pooled and again annealed in the second annealing to excess driver. During the first annealing, sequences common to both populations anneal. Additionally the concentration of high and low abundance messages are normalized since annealing is faster for abundant molecules due to the second order kinetics of hybridization. During the second annealing cDNAs unique or overabundant to the tester can anneal together. Such molecules have different adapters at their ends. The addition of additional driver during the second annealing enhances the enrichment of the desired differentially expressed sequences. During subsequent PCR, molecules that have different adapters at each end amplify exponentially. Molecules which have identical adapters, or adapters at only one end, or no adapters (driver sequences) either do not amplify or undergo linear amplification. The end result is enrichment for cDNAs corresponding to differentially expressed messages unique to the tester or up regulated in the tester.

This technique was used to identify transcripts unique to cancer tissues or messages overexpressed in the cancer process. To do this, pairs of samples isolated from a cancer tissue were used as the "tester", and non-cancer tissue as the "driver". The non-cancer "driver" can be from the same

individual and tissue as the tumor (Matched). Alternatively, the "driver" can be from a different individual but the same tissue as the tumor sample (unmatched). In some cases, the "driver" comprises mixtures of cDNAs derived from non-cancer tissues different from the cancer tissue. This approach allows the identification of transcripts whose expression is specific or up-regulated in the cancer tissue. Such transcripts may or may not be cancer specific in their expression.

Subtractive hybridization was carried out using as "tester" a mixture of three RNAs from human adenocarcinomas and as "driver" a mixture of RNAs from five human normal tissues (spleen, pancreas, heart, kidney and small intestine).

The subtracted mixture was cloned and two hundred clones were sequenced. One of the sequences matched Incyte clone ID 3966820. The electronic Northern for this clone showed the highest number of ESTs came from prostate, compared with other tissues (prostate 107 followed by uterus with 20).

The PCR-select clone detected a transcript of 1.9 kb by hybridization in Northern blots. Amongst 17 tissues tested prostate showed the highest abundance for this transcript.

Example 2: Relative Quantitation of Gene Expression

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'- 3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected

by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of the target gene were evaluated for every sample in normal and cancer tissues. Total RNA was extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probes specific to each target gene. The results were analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

Real-Time quantitative PCR was done using the following primers:

pro108 Reverse GCCTTCAGCCGTGGGTAGT (SEQ ID NO:3)

pro108 Forward GACAGCGGCTTCACCTTCTC (SEQ ID NO:4)

The absolute numbers depicted in Table 1 are relative levels of expression of the CSG referred to herein as pro108 (SEQ ID NO:1) in 11 normal different tissues. All the values

are compared to normal pancreas (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 1: Relative Levels of CSG Pro108 Expression in Pooled Samples

Tissue	NORMAL
Colon Ascending	4.56
Kidney	0.78
Liver	5.66
Ovary	41.07
Small Intestine	0.78
Spleen	3.29
Stomach	11.39
Testis	2.04
Uterus	15.56
Pancreas	1.00
Prostate	2.32

The relative levels of expression in Table 1 show the highest mRNA expression in ovary (41.07) and uterus (15.56), two female specific tissues. Except for stomach (11.39) that shows high levels of mRNA for pro108, the other tissues including prostate show comparable low levels of expression.

The absolute numbers in Table 1 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 2.

The absolute numbers depicted in Table 2 are relative levels of expression of the CSG pro108 in 70 pairs of matching samples. All the values are compared to normal pancreas (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Unmatched samples were used for prostatitis (prostatitis 1 and 2) and Benign Prostate Hyperplasia (BPH 1 to 6).

Table 2: Relative Levels of CSG Pro108 Expression in Individual Samples

Sample ID	Tissue	Cancer	Benign Diseases	Normal Adjacent Tissue
Pro12B	Prostate 1	253.40		7.70
Pro78XB	Prostate 2	1020.46		96.67
Pro84XB	Prostate 3	2055.11		70.52
Pro101XB	Prostate 4	872.61		218.58
Pro91X	Prostate 5	539.32		195.36
Pro13XB	Prostate 6	0.60		0.49
Pro23B	Prostate 7	929.30		747.00
Pro90XB	Prostate 8	160.50		21.00
Pro18XB	Prostate 9	45.63		31.49
Pro20XB	Prostate 10	312.50		20.00
Pro34B	Prostate 11	1351.18		142.02
Pro65XB	Prostate 12	1305.15		8.37
Pro69XB	Prostate 13	486.50		14.50
Pro10R	Prostatitis 1		122.45	
Pro20R	Prostatitis 2		83.38	
Pro258BPH	BPH 1		39.33	
Pro263CBPH	BPH 2		451.98	
Pro267ABPH	BPH 3		90.78	
Pro271ABPH	BPH 4		5.21	
Pro460ZBPH	BPH 5		15.64	
ProC032BPH	BPH 6		46.85	
Bld32XK	Bladder 1	2.86		3.88
Bld46XK	Bladder 2	15.62		1.45

Bld66X	Bladder 3	32.67		19.90
BldTR14	Bladder 4	330.60		235.50
ClnAS67	Colon 1	71.26		0.51
ClnRC01	Colon 2	5.08		25.90
ClnSG45	Colon 3	19.09		6.61
ClnSG67	Colon 4	65.12		20.61
ClnTX01	Colon 5	116.97		6.25
ClnB34	Colon 6	3.98		2.45
ClnB56	Colon 7	1.98		1.41
ClnC9XR	Colon 8	6.88		2.00
ClnCXGA	Colon 9	2.25		2.62
ClnAS89	Colon 10	77.44		2.39
ClnTX67	Colon 11	167.73		11.51
End10479	Endometrium 1	7.63		26.67
End8911	Endometrium 2	16.19		64.98
End8963	Endometrium 3	12.14		65.89
End5XA	Endometrium 4	99.39		23.51
End65RA	Endometrium 5	6.94		16.06
Kid109XD	Kidney 1	65.57		41.93
Kid10XD	Kidney 2	39.67		9.71
Kid11XD	Kidney 3	12.68		1.62
Kid126XD	Kidney 4	43.71		7.59
Kid12XD	Kidney 5	2.50		70.80
Kid5XD	Kidney 6	2.11		74.29
Kid6XD	Kidney 7	19.36		0.72
Liv42X	Liver 1	39.00		6.40
Liv15XA	Liver 2	9.23		4.59
Liv94XA	Liver 3	7.07		8.09

LngAC66	Lung 1	47.30		16.20
LngBR94	Lung 2	106.50		3.40
LngLC109	Lung 3	9.60		34.10
LngLC71	Lung 4	70.30		64.00
LngSQ56	Lung 5	12.40		53.80
LngSQ79	Lung 6	108.40		77.20
Lng60XL	Lung 7	0.55		1.47
Lng75XC	Lung 8	2.34		3.11
LngC17X	Lung 9	52.96		31.71
LngAC69	Lung 10	654.84		42.67
LngC20X	Lung 11	2.82		0.22
Mam47XP	Mammary Gland 1	3.40		1.89
Mam82XI	Mammary Gland 2	33.29		4.44
MamB011X	Mammary Gland 3	10.61		1.50
MamA06X	Mammary Gland 4	14.62		1.55
Ovr	Ovary	10.00		8.00
Pan77X	Pancreas 1	91.27		24.50
Pan71XL	Pancreas 2	1.55		1.30
Pan82XP	Pancreas 3	5.49		7.53
Pan92X	Pancreas 4	1069.00		688.40
StoAC99	Stomach 1	50.70		18.60
StoTA73	Stomach 2	37.70		130.30
SmI21XA	Small Intestine 1	12.42		5.31
SmIH89	Small Intestine 2	23.26		2.63
Tst39X	Testis	27.95		7.01

Utr85XU	Uterus 1	3.30		4.91
Utr23XU	Uterus 2	44.48		29.04

Table 2 shows the results for the analysis of 148 samples distributed in 14 different tissue types. Tables 1 and 2 represent a combined total of 159 samples in 16 different tissue types.

In the analysis of matching samples, the higher levels of expression were in prostate, showing a high degree of tissue specificity for prostate tissue. Of all the samples different from prostate which were analyzed (57 matching samples), only a few samples (lung 10 and pancreas 4) showed an expression comparable or higher than the median for the mRNA expression in prostate cancer (median: 539.32).

In addition, the level of mRNA expression in cancer samples was compared with the isogenic normal adjacent tissue from the same individual. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 2 shows overexpression of the CSG pro108 in 13 of 13 prostate cancer tissues compared with their respective normal adjacent (prostate samples #1 to #13). Thus, there was overexpression in the cancer tissue for 100% of the prostate matching samples tested.

Altogether, the level of tissue specificity, plus the mRNA overexpression in 100% of the prostate matching samples tested are indicative of the CSG pro108 being a diagnostic marker for prostate cancer.

What is claimed is:

1. A method for diagnosing the presence of prostate cancer in a patient comprising:

(a) measuring levels of CSG in cells, tissues or bodily fluids in a patient; and

(b) comparing the measured levels of CSG with levels of CSG in cells, tissues or bodily fluids from a normal human control, wherein a change in measured levels of CSG in said patient versus normal human control is associated with the presence of prostate cancer.

2. A method of diagnosing metastases of prostate cancer in a patient comprising:

(a) identifying a patient having prostate cancer that is not known to have metastasized;

(b) measuring CSG levels in a sample of cells, tissues, or bodily fluid from said patient; and

(c) comparing the measured CSG levels with levels of CSG in cells, tissue, or bodily fluid of a normal human control, wherein an increase in measured CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

3. A method of staging prostate cancer in a patient having prostate cancer comprising:

(a) identifying a patient having prostate cancer;

(b) measuring CSG levels in a sample of cells, tissue, or bodily fluid from said patient; and

(c) comparing measured CSG levels with levels of CSG in cells, tissues, or bodily fluid of a normal human control, wherein an increase in measured CSG levels in said patient

versus the normal human control is associated with a cancer which is progressing and a decrease in the measured CSG levels is associated with a cancer which is regressing or in remission.

4. A method of monitoring prostate cancer in a patient for the onset of metastasis comprising:

(a) identifying a patient having prostate cancer that is not known to have metastasized;

(b) periodically measuring levels of CSG in samples of cells, tissues, or bodily fluid from said patient; and

(c) comparing the periodically measured CSG levels with levels of CSG in cells, tissues, or bodily fluid of a normal human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

5. A method of monitoring a change in stage of prostate cancer in a patient comprising:

(a) identifying a patient having prostate cancer;

(b) periodically measuring levels of CSG in cells, tissues, or bodily fluid from said patient; and

(c) comparing the periodically measured CSG levels with levels of CSG in cells, tissues, or bodily fluid of a normal human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease is associated with a cancer which is regressing in stage or in remission.

6. The method of claim 1, 2, 3, 4 or 5 wherein the CSG comprises SEQ ID NO:1 or SEQ ID NO:2.

7. An antibody which specifically binds CSG.

8. A method of imaging prostate cancer in a patient comprising administering to the patient an antibody of claim 7.

9. The method of claim 8 wherein said antibody is labeled with paramagnetic ions or a radioisotope.

10. A method of treating prostate cancer in a patient comprising administering to the patient an antibody of claim 7.

11. The method of claim 10 wherein the antibody is conjugated to a cytotoxic agent.

SEQUENCE LISTING

<110> Ali, Shijath M.
Sun, Yongming
Salceda, Susana
Recipon, Herve
Cafferkey, Robert
DIADEXUS LLC

<120> A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Prostate Cancer

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23764

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 141.1, 155.1, 183.1; 435/6, 7.1, 7.23, 7.9, 91.2; 436/501, 504, 505, 547; 514/44; 536/ 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, Biosis, Embase, Cancerlit, Scisearch, WPIDS, USPATFULL
search terms: CSG, cancer specific gene, cancer, diagnosis, staging, prognosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OLSSON et al. CReverse tanscriptase-polymerase chain reaction assays for prostate cancer. Urologic Clinics of North America. May 1997. Vol.24, No. 2, pages 367-378.	1-5
Y	CHO-CHUNG. Antisense oligonucleotides for the treatment of cancer. Current Opinion in Therapeutic Patents. 1993. Vol.3, No. 12, pages 1737-1750.	1-5
A, E	BUSSEMAKERS et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Research. 01 December 1999. Vol.59, No 23, pages 5975-5979.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 FEBRUARY 2000	Date of mailing of the international search report 07 MAR 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GEETHA P. BANSAL Telephone No. (703) 305-3955

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23764

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 39/395, 48/00; C12P/ 19/34; C12Q 1/68; GO1N 33/53, 33/574, 33/546, 33/567

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 141.1, 155.1, 183.1; 435/6, 7.1, 7.23, 7.9, 91.2; 436/501, 504, 505, 547; 514/44; 536/ 23.5